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RESEARCH ARTICLE

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Regular monitoring and targeted removals can control lionfish in Mediterranean Marine Protected Areas

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Abstract

1. A lack of biosecurity in the Suez Canal has combined with global warming and other human pressures to cause abrupt changes in the Mediterranean Sea. Throughout this region an influx of species is influencing the outcome of efforts to protect and restore nature.
2. Despite calls for targeted removals of invasive species from protected areas, there is limited information about the effectiveness of this course of action from both an ecological and a socio-economic perspective. In this study, coordinated removals of lionfish (*Pterois miles*) by volunteers/scuba divers at three marine protected sites in Cyprus were conducted.
3. The removal efficiency was monitored using visual-census surveys and citizen science data. Removals significantly decreased lionfish numbers but long-term suppression of lionfish would require monitoring and repetition of removals when necessary, since population recovery was sometimes rapid.
4. Citizen science yielded the data needed to understand lionfish population changes and guide the timing of removal events, but was characterized by large variation and potential outliers, highlighting the need for large sample sizes.
5. Questionnaire surveys were used to assess the social impact of participation in lionfish removals; these showed that involvement had a strong positive impact on knowledge about lionfish and motivation to support marine conservation activities – the divers were even willing to pay extra to remove lionfish.
6. Management reforms would be needed to capitalize on this societal motivation, and enable effective lionfish removals by scuba divers, coordinated by competent authorities. The EU aims to protect at least 30% of the marine waters by 2030. Removal events could help shield selected conservation sites from the adverse effects of lionfish and at the same time help establish links with local communities, strengthening the sustainable use of marine systems both at corporate and at societal levels.

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KEYWORDS

alien species, citizen science, climate change, eradication, invasive species, management, non-indigenous species, *Pterois*, Suez Canal

1 | INTRODUCTION

Translocation of marine species beyond their native ranges is centuries old, but has been accelerating in recent years owing to increasing transcontinental shipping, aquaculture and ocean sprawl (Firth et al., 2016; Seebens et al., 2017). Some of these species can disrupt ecosystems, often assisted by changes in climate and human impacts on habitats (Chaffin et al., 2016; de Castro, Fileman & Hall-Spencer, 2017; Geburzi & McCarthy, 2018), overfishing of native predators and limited biotic resistance of the recipient ecosystems (Kimbrow, Cheng & Grosholz, 2013; Crocetta et al., 2021). Sometimes non-native species have beneficial effects, such as the provision of biogenic reef and the filtration of eutrophic water by oysters (Davis et al., 2011; Lemasson et al., 2017). The human introduction of lionfish (*Pterois* spp.) into the western Atlantic (Albins & Hixon, 2013; Côté & Smith, 2018) caused widespread negative effects such as reduction of native fish abundance (Green et al., 2012; Côté, Green & Hixon, 2013; Ballew et al., 2016) and a shift in benthic habitats in favour of macroalgae rather than corals (Lesser & Slattery, 2011).

Since 2016, lionfish have been spreading rapidly in the Mediterranean Sea (Kleitou, Hall-Spencer & Kleitou, 2016; Kleitou et al., 2019c). They arrived from the Red Sea via the Suez Canal with multiple subsequent introductions increasing the genetic diversity of the Mediterranean population (Bariche et al., 2017; Dimitriou et al., 2019). In just a few years, lionfish have become established in the Levantine Sea, the southern and central Aegean Sea and the Greek Ionian Sea, and individuals have reached Tunisia and Italy (Dimitriadis et al., 2020; Kleitou et al., 2021b); this is one of the fastest rates of spread of a Red Sea fish in the Mediterranean (Poursanidis et al., 2020). Lionfish in the Mediterranean have similar biological traits to those of the western Atlantic, such as generalist predatory behaviour, early maturity and rapid growth (Savva et al., 2020), combined with access to naive prey (Agostino et al., 2020).

Invasive species such as lionfish are spreading in areas designed to protect habitats and species from local stressors such as destructive development, fishing and pollution (Galil et al., 2017; Sala & Giakoumi, 2017). In the eastern Mediterranean, invasive species can be found in greater abundances in marine protected areas than in adjacent waters (Giakoumi et al., 2019b; D'Amen & Azzurro, 2020), so protected areas might end up providing refuges for invasive species with spillover and larval subsidy effects on adjacent areas (Galil, 2017; Corrales et al., 2018; Di Lorenzo et al., 2020).

Targeted removal has been suggested as a means of managing invasive species in marine protected areas (Giakoumi

et al., 2019a; Giakoumi et al., 2019b), but there is a lack of information on its ecological and socio-economic efficiency. As spearfishing has been effective for lowering lionfish numbers at selected locations in the western Atlantic (Barbour et al., 2011; De León et al., 2013; Johnston & Purkis, 2015; Chagaris et al., 2017; Harms-Tuohy, Appeldoorn & Craig, 2018; Harris et al., 2019), trials of this approach were organized in Cyprus where lionfish have started to become common in marine protected areas (Kleitou et al., 2019b). In this study, the efficiency of removal events was monitored using visual census of fixed transects on rocky habitats by researchers and by volunteer (i.e. citizen science) surveys on a shipwreck. A questionnaire was used to assess the social dimensions of such measures. The study aimed to assess:

1. the efficiency of involving volunteers in monitoring the populations of lionfish and guiding management interventions;
2. the efficiency of targeted removal events by volunteers in decreasing the lionfish numbers from marine protected areas; and
3. the socio-economic dimensions of the participation of the volunteers in lionfish removals.

2 | METHODS

2.1 | Training and implementation of removal events

From May to November 2019 five removal events were organized for volunteer divers to catch lionfish at three marine protected sites off Cyprus (*Chapel*, *Cyclops* and *Zenobia* wreck; Figure 1). For these events, divers were trained and formed Removal Action Teams for lionfish, following permission (special licence) obtained from the coastal police and the Department of Fisheries and Marine Research (Ministry of Agriculture, Rural Development and Environment) of Cyprus.

Specifically, three recurring events were conducted at Cape Greco, Larnaca and Limassol. The events were attended by 66 experienced divers; 55 were men and 11 were women. All participants were residents of Cyprus; 43 of them had Cyprus nationality. All participants had at least an Advanced Open Water Dive qualification or equivalent, and 30% were scuba instructors. During the workshops (Figure 2a), divers were informed about the lionfish invasion, biology, ecology and edibility of lionfish, its safe handling and the use of the removal toolkit (pole spears, containers and puncture resistant gloves) that was assembled by the project and approved by the Cyprus authorities.

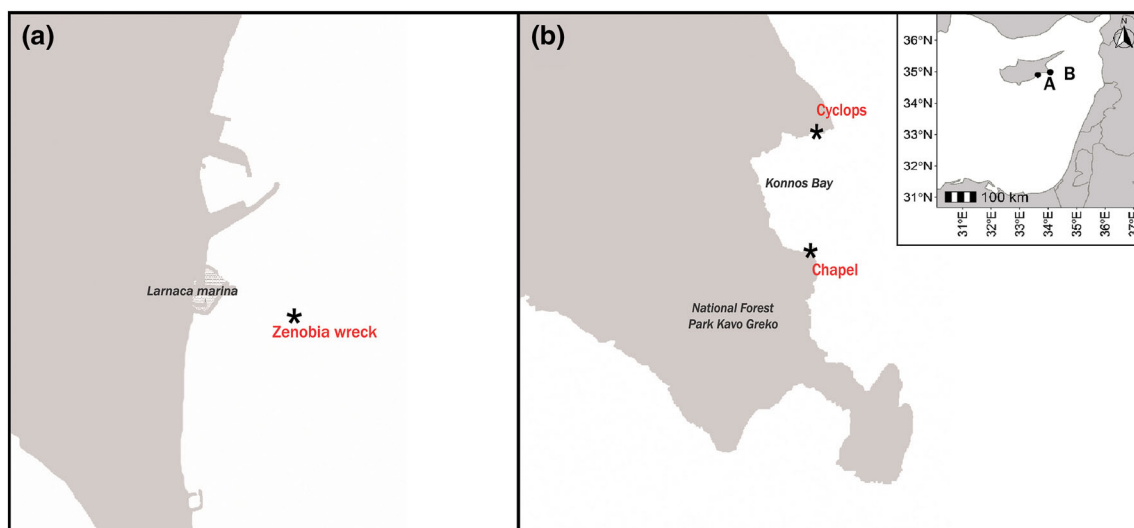


FIGURE 1 Lionfish removals were conducted by volunteers using scuba at three Marine Protected Sites off Cyprus in 2019 (one site at a, two sites at b). (a) Site of the *Zenobia* shipwreck off Larnaca, a no-fishing area. (b) The popular diving sites *Cyclops* and *Chapel* within Cape Greco Marine Protected Area

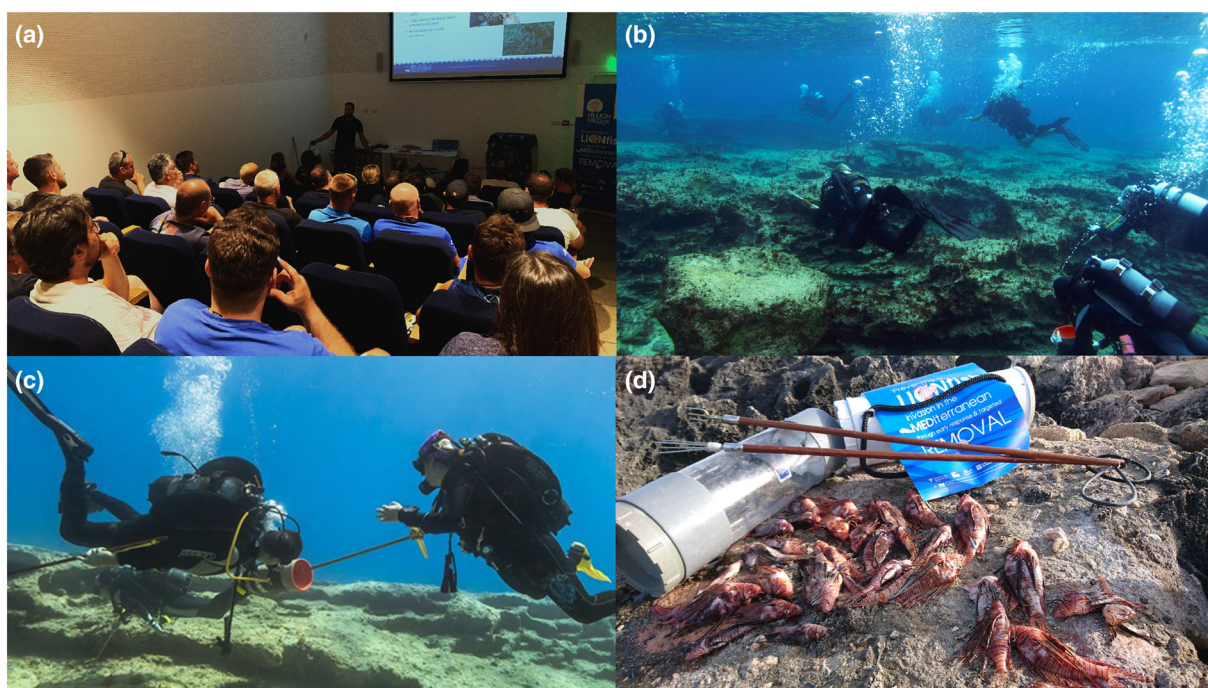


FIGURE 2 (a) Diver training event about lionfish and their safe removal that took place at Cape Greco Environmental Information and Education Centre on 25 May 2020; (b) groups of up to 18 divers worked together to remove lionfish, here at about 5 m depth on rocky reef habitat within Cape Greco Marine Protected Area at *Cyclops*; (c) each time a lionfish was speared it was held and removed using a special container for safe handling of multiple specimens (26 May 2019 at 10 m depth at *Cyclops*). Picture was provided by the Removal Action Teams member 'Pantelis Kranos' (Cyprus). (d) Spears and container with catch contents emptied onto the shore (6 June 2019 at *Cyclops*)

The efficiency of the removal events in reducing lionfish numbers and increasing public participation was monitored using three methods: citizen science, fixed transect monitoring and structured questionnaires. Following removal events, the specimens were provided to the participants for consumption.

2.2 | Citizen science monitoring of the *Zenobia* shipwreck

Fishing is prohibited on the *Zenobia*, a 172 m long, 28 m wide and 21 m high steel shipwreck lying on its starboard side on a level

muddy-sand sea bed at 42 m and the port side at 16 m depth off Larnaca (Figure 1a). The wreck is far (>4 km) from rocky and seagrass habitats that lionfish commonly use in the Mediterranean Sea (Savva et al., 2020). Lionfish were first seen at this regularly dived site in 2015 (Kleitou, Hall-Spencer & Kleitou, 2016). From May to December 2019, the divers were provided with logbooks and asked to report their *Zenobia* lionfish sightings via email, phone or social network platforms. They were asked to provide information about all lionfish observed on each of their dives on this wreck, along with dive duration, dive gear used, depth range of the dive, depth of lionfish sightings, habitat, bottom and surface temperature, time of the day, exact location of the dive, and any other qualitative information that they thought relevant. To standardize lionfish observed per unit effort, the number of lionfish seen per minute dive time (Observations per minute) was used. To correlate citizen science sightings and observations per minute effort, the Kendall's tau rank correlation coefficient was used. To avoid the effect of management interventions and measure the correlation between the lionfish sightings and bottom/surface temperature, the dataset was split into three sets: one with the data received before the first removal event; one with the data received between the first and second removal events; and one with the data received after the second removal event. The correlation between citizen sightings and bottom/surface temperature was examined for all three intervals using Kendall's tau rank correlation coefficient.

2.3 | Fixed transect monitoring in Cape Greco Marine Protected Area

To assess the efficiency of targeted removals, fixed transects were established and monitored at two sites set about 1 km apart in the Cape Greco Marine Protected Area (Figure 1b) where targeted removal events were conducted. *Cyclops* was rocky (Figure 2c, d) with boulders and small caves and crevices to 15 m depth with *Posidonia oceanica* meadows to over 35 m and then soft substrate. *Chapel* had steep rock to 10–15 m, followed by sandy expanses intermixed with hard substrata and patches of *P. oceanica*. During the removal events, the divers were free to move/swim in any direction and habitat of their choice, but they were restricted to an area of about 300 × 200 m at each site. At both sites, six 50 m long fixed transects were haphazardly established on hard substrata between 5 and 20 m in an effort to randomly distribute them over the targeted area of the divers. The transects of each area were monitored three times before and after the removal events.

Lionfish density and biomass were estimated using an underwater visual census method developed by Green et al. (2013) since it was found, after pilot studies, to detect lionfish more reliably compared with other techniques (Kleitou et al., unpublished data). Survey divers swam in a zig-zag pattern, searching crevices and overhangs (using a dive torch when needed) to record all lionfish 10 m either side of the transect line. For every lionfish recorded, its length was estimated *in situ*. Total length data were used to calculate fish

biomass using the equation $W = a \times L^b$, where W is the net mass (g) and L the total length (cm). Parameters a and b were based on Savva et al. (2020). The surveys were conducted by the same researchers at the same six strip transects in each site, prior to and shortly after the removal events, on 24 May 2019, 31 May 2019 and 12 June 2019.

Lionfish sizes, abundance and biomass were compared using a one-way repeated measures ANOVA (also known as a within-subjects ANOVA) for each of the areas. *Post hoc* comparisons were analysed using paired *t*-tests with a Bonferroni correction. The data were checked for significant outliers (boxplots), normality (Shapiro-Wilk normality test and quantile-quantile plots), homogeneity of variance (model residuals plot and Bartlett test) and homogeneity between the repeated measures (Mauchly's test, $P = 0.002$). When assumptions were not met (i.e. biomass data at *Chapel*), square root transformation was applied. For all statistical analyses a significance level was set at 0.05, and their computation was carried out using R-Studio (v 1.2.1335).

2.4 | Monitoring the social dimension of removal events

Questionnaires were carried out face-to-face with 25 random participants during their first participation at the training or removal events before they received the caught fish. They were designed to assess their knowledge about lionfish, their motivation to be involved in marine invasive species conservation activities and willingness to pay a fee to observe lionfish, participate in removal activities or support efforts in controlling lionfish. Specifically, 11 questions were asked as shown in Table 1. All interviews were carried out by the same trained person, ensuring that questions were presented in an identical manner, and that prompts or influences were similar across all interviewees. The encounters were held privately, in one-to-one sessions, to prevent influence or interference by other people. To avoid distrust, respondents were approached informally and asked if they were willing to answer a few questions about their participation in the events. The responses about the willingness of divers to pay extra for a dive to observe/find, remove or support others in controlling lionfish were binned into two nominal categories: not pay and pay a fee (from €1 to >€10), and tested for equal proportions using a chi-square goodness of fit test for each statement.

3 | RESULTS

3.1 | Removal events

Removal events went smoothly, helped by the fact that the volunteers were experienced divers operating in warm waters with minimal currents and exceptionally good underwater visibility compared with most coastal environments (Figure 2b, c). Between 35 and 119 lionfish were removed per day by nine to 27 divers at

TABLE 1 Questions used (in Greek and in English) to assess knowledge and attitudes amongst volunteers involved in lionfish removal events

Questions	Possible answers
<i>Part A: Impact of divers participation in removal events</i>	
On a scale of 0 to 10, where 0 = strongly disagree and 10 = strongly agree, to what extent did the removal events helped or encouraged you to:	Scale: ranking order of preference 0–10, where 0 = strongly disagree, 5 = neutral, and 10 = strongly agree
1. Support potential management measures against invasive species?	
2. Collaborate with scientists and management authorities?	
3. Participate in conservational activities?	
4. Understand lionfish potential ecological and socio-economic impacts?	
5. Understand that lionfish is edible?	
<i>Part B: Willingness to pay extra fee in a dive</i>	
Would you pay extra fee to:	Multiple choice:
1. Observe lionfish underwater in the Mediterranean?	(a) No, I would not pay extra
2. Participate in a dive and remove lionfish in the Mediterranean?	(b) I would pay €1 extra for the dive
3. Support others (e.g. management authorities) in controlling the lionfish in the Mediterranean?	(c) I would pay €2–5 extra for the dive
	(d) I would pay €6–10 extra for the dive
	(e) I would pay more than €10 for the dive
<i>Part C: Socio-demographic information</i>	
Gender	Dichotomous: male/female
Age	Multiple choice:
	(a) 18–24
	(b) 25–34
	(c) 35–44
	(d) 45–54
	(e) 55–64
	(f) Over 65
Nationality	Open-ended question

each protected site (Figure 2b–d, Table 2). The catch efficiency (percentage of lionfish caught/lionfish detected) ranged between 56.92 and 83.22% (Table 2). The catch per unit effort (CPUE) was lower at the *Zenobia* wreck compared with the two rocky sites where fewer dives were conducted (Table 2). Both CPUE and catch efficiency decreased after a removal event (Table 2).

3.2 | Citizen science monitoring of the *Zenobia* shipwreck

Citizen science dive records from the *Zenobia* ($N = 104$) provided lionfish sightings on 58 days out of a 233-day monitoring period that started on the 27 April 2019. Most records (88%) were sent via email with filled data logbooks, followed by communication via social networks (10%) and 3% via telephone. All of these dives were carried out between 09:00 a.m. and 13:30 p.m. The maximum dive depth of the dives ranged from 23 to 42 m. According to the additional qualitative information provided by the divers, lionfish were not that common inside the wreck and very dark places, with reports received such as ‘No lionfish inside the wreck’ and ‘Most lionfish were on outside, but a couple were inside in the twilight areas’.

Based on the citizen science records, lionfish numbers peaked in May–July 2019 prior to the first removal event (e.g. 58 lionfish observed in a single dive on 9 May 2019). The observations per dive minute correlated significantly with the total number of lionfish observed on dives (Kendall's tau = 0.62, $P < 0.05$, Figure 3a). Both fell sharply after removal events; especially after the first one (Figure 3a). Lionfish numbers did not completely recover for at least three months after the first removal. Owing to large variations, it is not clear whether the drop in the lionfish observations after the second removal was natural (e.g. a consequence of the observed temperature decrease) or due to the removal event, and more sightings were needed for valid conclusions.

Dive computers provided detailed *in situ* temperature data, showing clear thermal stratification of the water column from May to October and uniform temperature–depth profiles after a breakdown of the thermocline in November–December (Figure 3b). The surface temperature did not correlate with the lionfish observations received prior to the first removal event (Kendall's tau = 0.0022, $P > 0.05$), between the first and second removal events (Kendall's tau = 0.12, $P > 0.05$) and after the second removal event (Kendall's tau = 0.18, $P > 0.05$). Similarly, the bottom temperature did not correlate with the lionfish observations received prior to the first removal event (Kendall's tau = −0.039, $P > 0.05$), between the first and second

TABLE 2 Lionfish removals by volunteers at three marine protected sites off Cyprus in 2019 showing dates and numbers of divers, dives, lionfish removed, Catch Per Unit Effort [CPUE [number of lionfish caught/(number of divers × number of dives)], lionfish seen but not caught and percentage catch efficiency (number of lionfish removed/lionfish seen)

Site	Removal event date	Number of divers participating	Number of dives conducted	Lionfish removed	CPUE	Lionfish missed	Catch efficiency (%)
<i>Cyclops</i>	26 May 2019	18	1	72	4.00	38	65.45
	06 June 2019	11	1	35	3.18	21	62.50
<i>Chapel</i>	26 May 2019	9	1	38	4.22	16	70.37
<i>Zenobia</i> wreck	15 July 2019	22	2	119	2.70	24	83.22
	24 November 2019	27	1	37	1.37	28	56.92

Note: The CPUE and catch efficiency values are coloured according to the percentile of their category (green for percentile >50, white for 50 and red for <50).

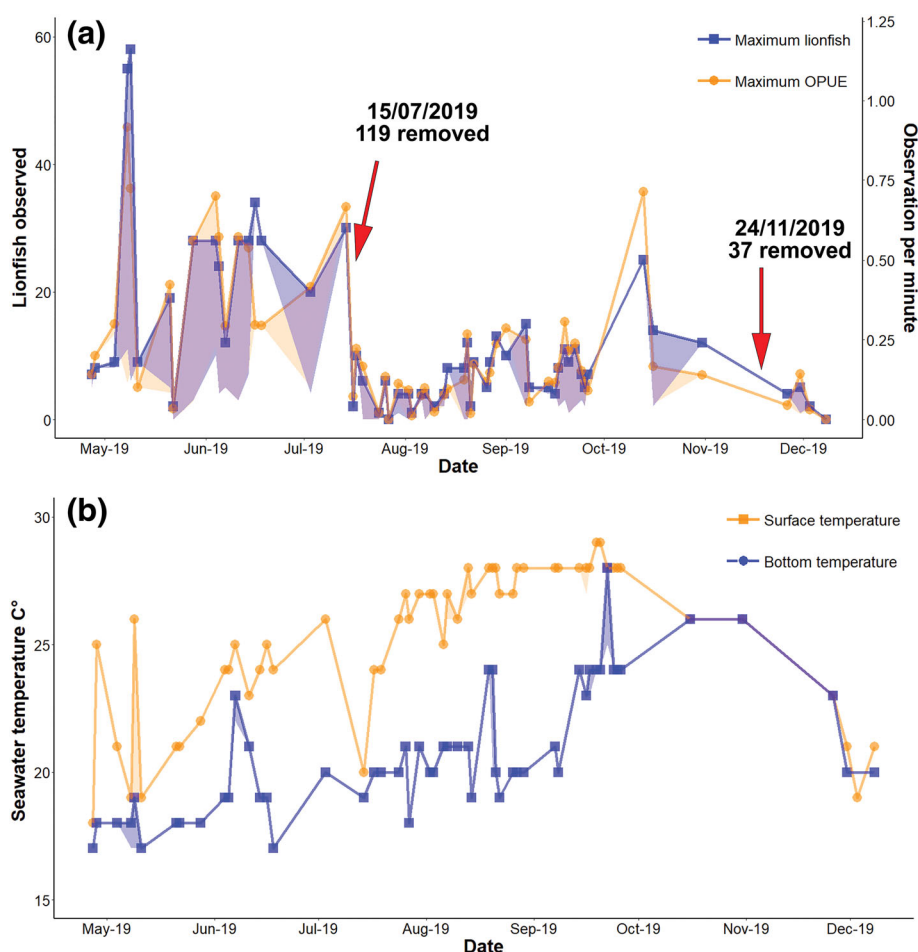


FIGURE 3 (a) Highest daily number of lionfish observed (blue) and highest dive observations per minute (OPUE) (orange) by volunteers on the *Zenobia* wreck, Cyprus in May to December 2019. Accordingly, the blue and orange shades indicate the lowest daily records of observations and OPUE (when more than one dive record was received). Red arrows show removal events. (b) Average bottom and surface seawater temperatures provided by scuba divers using their dive computers on the *Zenobia* wreck, Cyprus, May to December 2019

removal events (Kendall's tau = 0.12, $P > 0.05$) or after the second removal event (Kendall's tau = 0.82, $P > 0.05$).

3.3 | Fixed transect monitoring in Cape Greco Marine Protected Area

As with citizen science records of lionfish numbers per dive, visual census of fixed transects also revealed that lionfish abundance

decreased after removals, but the transect surveys were also able to estimate changes in lionfish abundance and biomass per unit area. Lionfish abundance at *Cyclops* decreased significantly over the series of removals (one-way repeated measures ANOVA [$F(2,10) = 6.22$, $P < 0.05$, $\eta^2 = 0.50$] from 10.5 ± 1.28 individuals per 1,000 m² before the removal events to 6.66 ± 1.74 individuals per 1,000 m² after one removal, and to 3.5 ± 0.43 individuals per 1,000 m² after two removals (Figure 4). Lionfish biomass at *Cyclops* decreased by about 50% after the initial removal event, although this was not statistically

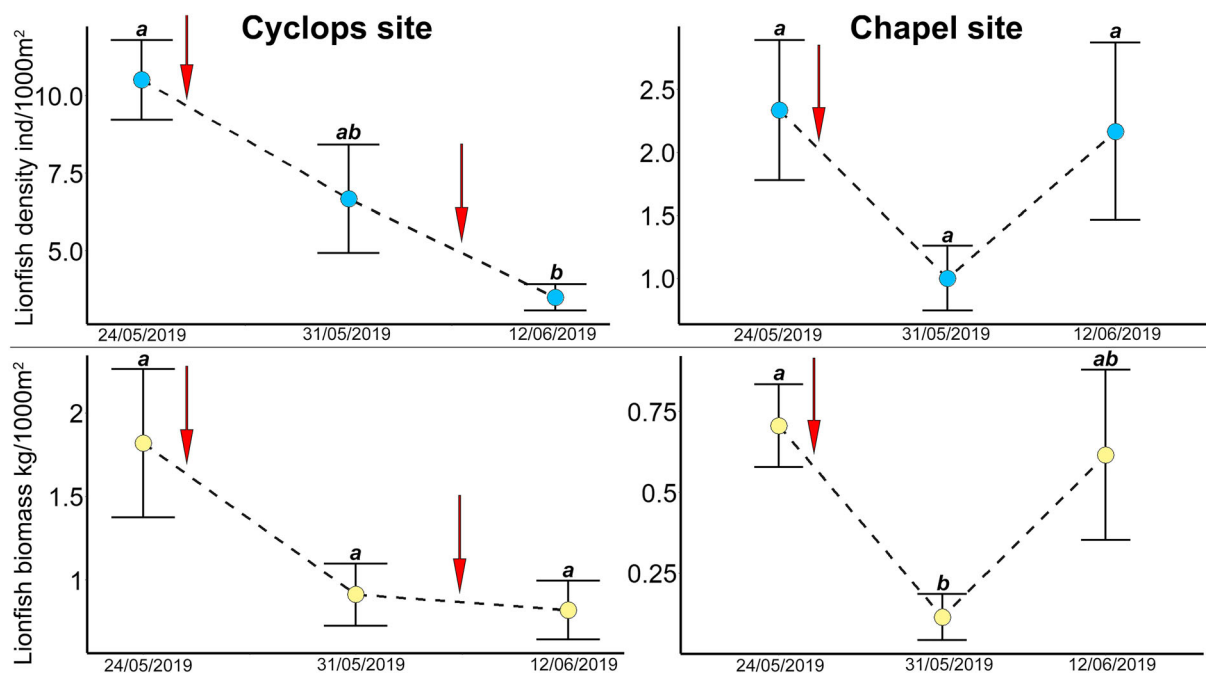


FIGURE 4 Average (\pm SE, $n = 6$) lionfish density and biomass of lionfish at two sites (Cyclops and Chapel) in Cape Greco marine protected area, Cyprus, 2019. Red arrows indicate removal events. A total of 72 lionfish were removed (38 missed) by 18 divers in the first removal at Cyclops on 26/05/2019, and 35 were removed (21 missed) by 11 divers in the second removal on 6 June 2019. At Chapel, 38 lionfish were removed (16 missed) by nine divers on 26 May 2019. Surveys that do not share a letter are significantly different at $P < 0.05$ (paired t -tests with a Bonferroni correction)

significant [one-way repeated measures ANOVA [$F(2,10) = 3.49$, $P > 0.05$, $\eta^2 = 0.32$]] as only a few transects ($n = 6$) could be used owing to logistical constraints on manpower, reducing the ability to detect statistically significant changes. The size of lionfish did not change significantly between sampling events [ANOVA [$F(2,10) = 1.13$, $P > 0.05$, $\eta^2 = 0.16$]] and ranged from 14.53 ± 2.58 cm in the first sampling to 14.54 ± 6.17 cm in the second with a slight increase to 17.91 ± 1.96 cm in the third owing to an increase in records of lionfish in the range of 20–25 cm (Figure 5).

At Chapel, lionfish abundance was much lower overall, and although it decreased after a removal event (2.33 ± 0.56 to 1 ± 0.63 individuals per 1,000 m²), this did not vary statistically over the surveys [one-way repeated measures ANOVA [$F(2,10) = 2.57$, $P > 0.05$, $\eta^2 = 0.19$]]. On the other hand, biomass dropped significantly [one-way repeated measures ANOVA [$F(2,10) = 5.38$, $P < 0.05$, $\eta^2 = 0.19$]], reflected by the second survey (paired t -tests with a Bonferroni correction, $P < 0.05$), which was preceded by a removal event (Figure 4). There was a significant shift in the size of lionfish at Chapel [ANOVA [$F(2,10) = 4.99$, $P < 0.05$, $\eta^2 = 0.33$]] after the removal event, which dropped from an average of 22.05 ± 4.69 cm in the first sampling to 10.58 ± 8.66 cm in the second and increased to 15.77 ± 8.59 cm in the third (Figure 5). Within the 12 days that intervened between the second and third surveys when no removal event took place, lionfish were able to almost recover their numbers (on a daily rate of increase of 0.97 lionfish individuals per hectare).

3.4 | Social aspects of removal events

Of the 25 participants who took part in face-to-face questionnaires, the majority were men (80%). Responses were taken across a well-distributed adult age range, with two being 18–24, six being 25–34, six being 35–44, four being 45–54, and six being 55–64 years. One respondent did not report their age. About half of them were Cypriots (52%, $n = 13$) followed by British (35%, $n = 8$).

According to these divers, their participation in the lionfish training and removals improved their knowledge about lionfish and motivated them to support management efforts. None of the participants reported negative effects of involvement on their motivation and knowledge (Figure 6). In all questions, more than 80% of the respondents reported positive impact (Likert scale score = 6–10) owing to their participation (median = 10; Figure 6) and that the removal events strongly encouraged them (Likert scale score = 10) to support other management measures against invasive species (71%, $n = 17$), collaborate with scientists and managers (70%, $n = 16$), participate in conservation activities (70%, $n = 16$), understand lionfish potential impacts (68%, $n = 15$) and understand that lionfish are edible (59%, $n = 13$) (Figure 6).

The willingness of divers to pay was negative when asked to dive to observe lionfish as the majority (80%) were not willing to pay at all (Pearson's chi-squared test, $\chi^2 = 9$, d.f. = 1, $P < 0.05$). On the other hand, divers were willing to pay to remove lionfish (Pearson's chi-squared test, $\chi^2 = 8.33$, d.f. = 1, $P < 0.05$), specifically 78% would

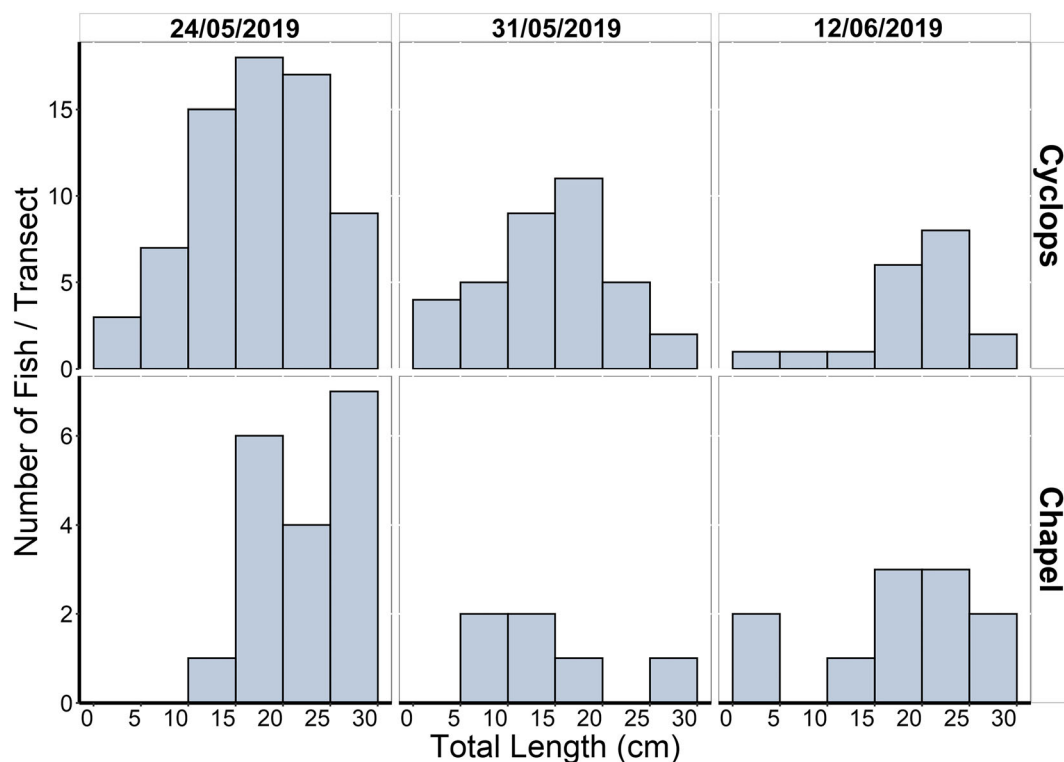


FIGURE 5 Length frequency histogram of the lionfish observed at *Cyclops* and *Chapel* in each of the visual census monitoring surveys

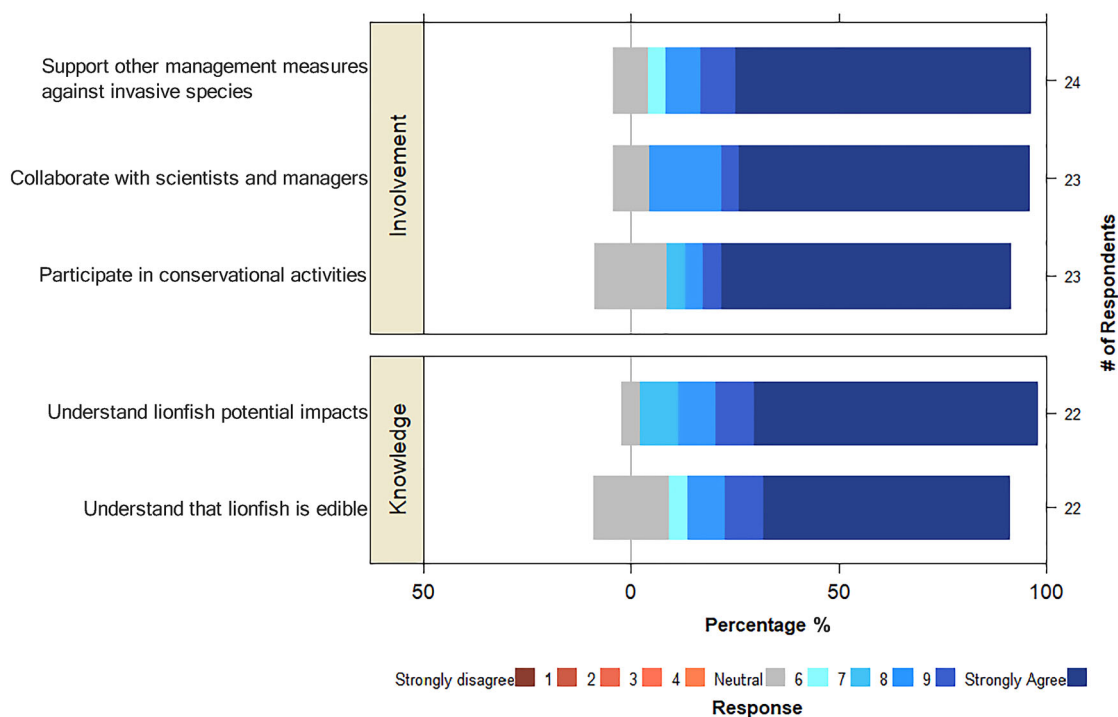
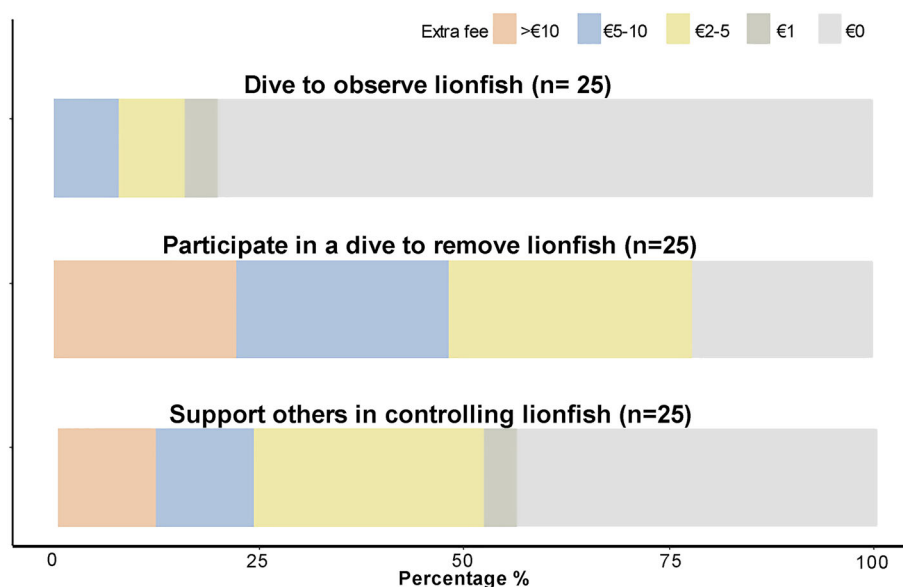


FIGURE 6 Agreement of divers from Cyprus about the effect of their participation in removal activities on their involvement and knowledge about lionfish. Proportions were acquired based on the categorization of the ordinal scores (0–10) to disagree (0–4), neutral (5) and agree (6–10)

FIGURE 7 Percentage divers from Cyprus asked if they would be willing to pay extra to (a) dive to observe lionfish, (b) participate in a dive to remove lionfish and (c) support other people in controlling lionfish



pay at least €2 extra to remove lionfish, 26% to pay at least €5 and 22% reported that they would be willing to pay €10 extra (Figure 7). When they were asked about supporting others' efforts in controlling lionfish, responses whether to pay or not were statistically similar (Pearson's chi-squared test, $\chi^2 = 0.36$, d.f. = 1, $P > 0.05$).

4 | DISCUSSION

In 2015, a 35 km long section of the Suez Canal was deepened and expanded from 61 to 312 m wide. This doubled shipping capacity and decreased transit time from 18 to 11 h for most vessels, which pay around \$450,000 per trip to use this waterway. Galil et al. (2015) highlighted the biosecurity dangers of this expansion, and the need for cost-effective mitigation strategies, since the canal was already one of the most potent corridors for marine species invasions in the world. In 2016, an incipient lionfish invasion was first noted in the region, leading to urgent calls for improved Suez Canal biosecurity (Kleitou, Hall-Spencer & Kleitou, 2016). Within just four years, lionfish from the Red Sea had become established over such a wide area that eradication was not feasible (Kleitou et al., 2019c; Booy et al., 2020).

This study drew upon experiences gained in dealing with invasive lionfish in the western Atlantic (Frazer et al., 2012; Usseglio et al., 2017). There, it has been shown that removal efforts with divers can be effective at suppressing lionfish populations in localized areas (Barbour et al., 2011; De León et al., 2013). Using biomass production of lionfish prey and rate of prey consumption by lionfish, Green et al. (2014) developed a size structured simulation model and predicted threshold damaging densities of lionfish beyond which native fish biomass starts to decline, indicating that removal efforts without complete eradication could be effective in helping preserve/restore the native biota. Similarly, Chagaris et al. (2017) used a trophic dynamic model and have shown that even relatively low levels of

lionfish harvesting can be translated into increases in the biomass of the rest of the community.

The successful removal events used in the Caribbean were replicated, and this study explored whether they could work in the socio-economic and environmental context of Mediterranean protected areas. It is illegal to spearfish with scuba in all Mediterranean countries (Gaudin & De Young, 2007), so a derogation from the government was given agreeing that a small number of well-trained divers could be involved in the trial programme. The results of this first attempt to address the spread of lionfish in the Mediterranean could be pivotal for the management authorities of countries where the lionfish has already invaded (i.e. Cyprus, Greece, Israel, Italy, Lebanon, Libya, Syria, Tunisia and Turkey).

Lionfish removal kits were assembled to furnish dive teams with the required handling and removal equipment. Training events were then followed by dives, attended by groups of nine to 27 divers, who removed up to 119 lionfish in a single day from selected marine protected areas. The participants engaged with the project enthusiastically and, on average, caught about 67% of the lionfish that they saw. The study has shown that diver-volunteers could play a critical role in Mediterranean lionfish management, supporting monitoring and reducing lionfish numbers at target sites. Involvement by citizens was also socially beneficial since according to the divers it increased their knowledge and encouraged their participation and collaboration in conservation.

The rocky habitat fixed transect monitoring and shipwreck citizen science surveys showed that removals decreased lionfish numbers within the Marine Protected Areas surveyed. Although these data showed large impacts of the removals on both abundance and biomass of lionfish, the decline was not always statistically significant. This can be attributed to factors such as low statistical replication, absence of control (i.e. no removal) sites (Underwood, 1992), different capacities of diver-volunteers in removing lionfish, and divers

targeting or focusing on large lionfish and potentially neglecting smaller individuals; thus there were cases where biomass was statistically reduced but the abundance was not. Despite the absence of control sites, it was evident that the decrease in lionfish populations was not due to natural variability but to the removal events, especially considering the short intervals between sampling events and the fact that lionfish are characterized by very high site fidelity and consistent site population densities (Jud & Layman, 2012; Akins, Morris & Green, 2014; Tamburello & Côté, 2015; Bos, Grubich & Sanad, 2018). The citizen-science shipwreck survey provided more updates as the *Zenobia* was dived regularly by the volunteers, confirming the ability of citizen science to collect vast amounts of data in a cost-effective manner. Common challenges faced by citizen-science projects such as misidentifications and poor data quality (Giovos et al., 2019) were potentially overcome by the fact that volunteer divers were trained and experienced, and that lionfish can be easily distinguished from other taxa owing to their conspicuous characteristics. Social media networks are effective at recording the spread of invasive species in Mediterranean countries (e.g. Gerovasileiou et al., 2017; Chartosia et al., 2018; Kleitou et al., 2019a; Kousteni et al., 2019), but tend to lack the detail needed to accurately estimate population levels. In our study, they have been found effective in helping understand the trends of populations and guiding management interventions, especially at isolated sites such as shipwrecks where data are more standardized. The electronic log-books yielded the data needed to guide the timing of removal events, although interpretation was needed – for example lionfish were much more common outside the wreck than within it, so data from teams that focused on exploring the wreck interior reported low numbers.

Using the sightings received by volunteers, large fluctuations in lionfish records were observed even within the same days and observations could be influenced by a range of factors such as the profile of/reason for the dive (e.g. explorative, instructional, etc.), observer, area of wreck explored, time of the dive, environmental conditions, etc. In days when more than one dive record was received, the use of the one with the maximum number of lionfish was considered as the most reliable that dealt better with detectability. The variation in observations highlights the importance of big sample sizes in citizen science monitoring. The observations per dive minute were correlated significantly with the total lionfish observed, indicating that standardization with unit effort (i.e. dive time) might not be a prerequisite in citizen science initiatives targeting isolated and remote areas such as shipwrecks. However, the collection of data that can enable standardization of citizen science dives, like dive duration, together with additional data such as the temperature, approximate area/location, time and the reason for the dive, is strongly recommended since it can provide useful information for understanding the changes that are observed.

Lionfish population recovery rates after removals (either from spillover/arrival of large individuals or larval subsidies from adjacent areas) varied amongst the study areas and should be taken into consideration in management efforts since they are related to the effort required for achieving significant conservation effects. Keeping

lionfish numbers below threshold damaging densities (Green et al., 2014) would require monitoring with removal events organized to deal with rising numbers of fish. For instance, relatively low initial lionfish numbers were able to recover to near pre-removal levels in 2 weeks in the areas of the fixed transects, while high initial lionfish numbers did not recover for at least 3 months after the first removal on the *Zenobia* wreck. Different recovery rates could reflect habitat connectivity; interconnected rocky habitat might allow spread from adjacent sites and so recovery can be rapid, whereas the *Zenobia* wreck was at least 4 km from the nearest rocky and seagrass habitats that lionfish commonly use in the Mediterranean Sea (Savva et al., 2020), which could explain the slower population recovery. In addition, the isolation of the wreck could imply that recruitment was primarily through larval settlement as opposed to the other two sites where immigration of larger fish from connected areas could more easily occur. The latter was confirmed by the length frequency of lionfish, which indicated that large lionfish individuals were re-introduced, especially at *Cyclops*. At *Chapel*, the number of large individuals decreased substantially, which suggests that they were targeted by the divers.

A trade-off between effort spent removing and the achievement of a smaller lionfish density was identified, as shown by the framework developed by Usseglio et al. (2017). The higher removal effort (44 dives) at the isolated *Zenobia* wreck was characterized by lower CPUE compared with the removal events of the other areas where fewer dives were conducted (<20 in each event). The CPUE further decreased to relatively low levels in the last removal event, indicating a potential depletion effect, justified by the slow recovery of lionfish numbers. Therefore, even one or two big removal events each year could be enough to protect remote sites such as the *Zenobia* wreck. On the other hand, the CPUE of the rocky sites was 1.5–2.5 times higher, indicating that more intense and/or frequent effort was required to achieve depletion effects. In addition to the decrease in CPUE, the catch efficiency of lionfish also decreased after each removal event. Anecdotal reports by the participants suggested that lionfish became alerted and more difficult to catch after removal events. A similar phenomenon was observed in the western Atlantic and should be taken into account as it can have implications for the impact of the invasive species and for the design and success of management measures (Côté et al., 2014).

The results of this study indicated that removal events can be effective in suppressing lionfish populations in targeted locations; however, long-term and larger-scale monitoring is needed to accurately understand the effects of site features such as connectivity and complexity, and decisively estimate the minimum effort that is needed to efficiently achieve depletion or suppression of lionfish populations below damaging levels. In addition, targeted removals by scuba divers are usually conducted in recreational depths of <30 m, and management efforts could be undermined by populations in deeper waters where individuals can be larger and consequently more fecund (Andradi-Brown et al., 2017). In the western Atlantic, specialized traps and harvesting robots targeting lionfish have been developed to target deeper populations (Harris et al., 2020; Strickland

et al., 2020), and their usage could be tested and promoted in the Mediterranean Sea.

High costs hinder the success of many invasive species control programmes worldwide, leading to temporary results (Britton, Gozlan & Copp, 2011; Pluess et al., 2012). Management reforms would be needed to ensure there was systematic and long-term commitment to lionfish removals (Kleitou et al., 2021a). In our study, divers were willing to pay an extra fee to participate in removal events or support others in removing lionfish. Special licences issued for protected areas to enable removal activities to be conducted and monitored by competent authorities/people can ensure that illegal activities such as spearfishing for grouper can be avoided. Similar mechanisms exist in other parts of the world. For example, Bonaire has a well-established marine conservation programme, the main body of which is run by the national park authority, and charges non-resident visitors a dive fee of \$45 per calendar year for scuba diving and \$25 for other water activities. Actions funded by this fee include a lionfish hunting programme, patrols to enforce fishing restrictions and coral reef monitoring (Roberts, Cresswell & Hanley, 2018). Bermuda is running a programme in which interested local volunteers are trained and receive an annual permit for lionfish removals while they can adopt a section of reef to regularly visit and cull lionfish (Gleason & Gullick, 2014). Hunting lionfish for consumption needs to be widely promoted as an ethically correct choice with added benefits to the ecology and environmental health (Noll & Davis, 2020).

In line with global targets to restore the ocean, the European Union aims to protect at least 30% of its marine waters, with one-third strictly protected by 2030 (EC, 2020; Laffoley et al., 2020). Marine Protected Areas are vulnerable to the spread of invasive species, and no-fishing zones are especially vulnerable to the spread of invasive fish such as lionfish (Galil, 2017). Citizens could play a pivotal role in monitoring and managing the species. Permitting divers to remove these fish using scuba gear will need to be applied with caution and strictly regulated to avoid illegal fishing. If implemented correctly, removal events could protect selected areas from the adverse effects of lionfish, while at the same time help to establish rich and deep links with local communities, strengthening responsibility and surveillance at corporate and societal levels, and stimulating public environmental awareness.

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